



VH G250

Figure 1

D V K L V E S G G G L V K L G G S L K L
GAC CTC AGC CTC GTG GAG TCT GGG GGA GGC TTA GTG AAG CTT GGA GGG TCC CTG AAA CTC
1 GAC CTC AGC CTC GTG GAG TCT GGA TTC ACT TTC AGT AAC TAT TAC ATG TCT TGG GGT CGC CAG ACT
S C A A S G F T P S N Y M S W V R Q T
61 TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AAC TAT TAC ATG TCT TGG GGT CGC CAG ACT
H1

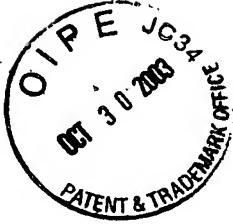
P E K R L E L V A A I N S D G G I T Y Y
GCC ATT ATT AGT GAT GGT GGT ATC ACC TAC TAT
121 CCA GAG AAG CGT CTG GAG TGT GTC GCA CGA ATT TCA AGA GAC ATT GCC AAT GCA AGC ACC CTG TAC
L D T V K G R F T I S R D N A K N T L Y
181 CTA GAC ACT GTC AGG GGC CGA ATT GCA GAG TCT GAC TAC
H2

L Q M S S L K S E D T A L F Y C A R H R
D T V K G R F T I S R D N A K N T L Y
L D T V K G R F T I S R D N A K N T L Y
181 CTA GAC ACT GTC AGG GGC CGA ATT GCA GAG TCT GAC TAC
H3

S G Y F S M D Y W G Q G T S V T V S S
TCG GGC TAC TTC TCT ATG GAC TAC TGG GGT CTA GGA AAC TCA GTC ACC GTC TCC TCA
301 TCG CAA ATG AGC AGT CTC AAG TCT GAG GAC ACA
H3

CDRs H1, H2, H3.

CDR definition according to Kabat scheme



VL G250

D I V M T Q S Q R F M S T T V G D R V S
1 GAC ATT GTG ATG ACC CAG TCT CAA AGA TTC ATG TCC ACA ACA GTA GGA GAC AGG GTC AGC

I T C K A S Q N V V S A V A W Y Q Q K P
61 ATC ACC TGC AAG GCC AGT CAG ATG GCT GTT TCT GCT GTC ACT GCA GGT TGG TAT CAA CAG AAA CCA

L1

G Q S P K L I Y S A S N R Y T G V P D
121 GGA CAA TCT CCT AAA CTA CTG ATT TAC TCA GCA TCC AAT CGG TAC ACT GGA GTC CCT GAT

L2

R F T G S G S G T D F T L T I S N M Q S
181 CGC TTC AGA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATT AGC AAT ATG CAG TCT

E D L A D F F C Q Q Y S N Y P W T F G G
241 GAA GAC CTG GCT GAT TTT TTC TGT CAA CAA TAT AGC AAC AAC TAT CCG TGG AGC TTC GGT GGA

L3

G T K L E I K
301 GGC ACC AAG CTG GAA ATC AAA

CDRs L1, L2, L3
CDR definition according to Kabat scheme

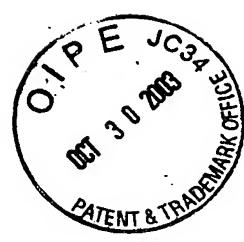


Figure 2

Primers used for PCR amplification of G250 VH and VL regions

5

Anchor and anchor poly C primers:

Anchor: 5'-GCA TGC GCG CGG CCG CGG AGG CC-3'

10 Anchor poly C: 5'-GCA TGC GCG CGG CCG CGG AGG CC(C)₁₂-3'

Constant primers:

15 VH-primers: 5'-CTC TAA GCT TGG CTC AAA CAC AGC
GAC CTC GGA TAC AGT TGG TGC AGC-3'

VL-primers: 5'-CTC TTC TAG AGA GTC TCT CAG CTG
GTA GGA TAC AGT TGG TGC AGC-3'



Figure 3A

08.05.2001

double strand sequencing

HC_cG250 (EcoRI-fragment in expression vector)

1 TCATGACATT AACCTTAAATAAGGGTA TCACGGGCC CTTCGTCCT CAAGAACATCTCAGATACAA AGAACATCTCA AACCCCTGAGG ACATTCCTATC 100
 101 ACAAAATAGT AAATTCAGA AAATTCGA TGCTCCATC ACAGAGATGA ATCTGCTATG AACAGCTCAT AGGTGTGAG CTCTACAAA GCCATATTAT 200
 201 TGAAGGCCA CATTGTGCCG AGACTTGGG AAGACTGAGC TCATATCCTG AAAATACATT ATGTGTGTT CTATCTAAT ACACATTAC ACTAAGGAAA 300
 301 CATGGCAGTA TGGGAATGAA CCTTGTCTG TACACATTA CAGGGGAA CTAACAAAG TATGGTGTAT CCCTAACCA AAGTAAAAAA AAAAARAAA 400
 401 AGAAAGAAA AGAAAGAAA AAGTGAAACT ACAATATGTT TCAAATGCTG TAATGAAAT CTGGTGTCTT GATGCCCTAT ATCTGTTATC ATCAGTGACT 500
 501 TCAGATTTAG TCCAACTCCA GAGCATGGTA TAGCATGGG ACATGCAAAT AGGTCTCTC TGTGCCCATG AAAAACACT CGGCCCTGAC CGTGAGCTC 600
 601 TGACAGAGGA GGCTGTCTT GGATTGATT CCATTCCT CACATTGAT GATCAGCACT GAAACAGAC CCCTCACCAT GAACTCGGG CTCAAGATTA 700
 701 TTTCCTCTGT CCTGGTTTA AAAGGTATCT TATTGAGTAT AGAGGACATC TGCTGTATGC ACAGAGGTGC AGAAAGAATG TTCTTGTTT GTTGTAGTGA 800
 801 CAATGCTCAGAACAGTATIC TTCTTTCGA GGTGTCTCTGT GTGACCTGAA GCTTGTGGAG TCTGGGGGA GCTTGTAGTGA GCTTGTGGGG TCCCTGAAAC 900
 901 TCTCCCTGTC AGCCCTCTGGA TCTCACTTCA GTCAGCTTCA GTCAGCTTCA GTCAGCTTCA GTCAGCTTCA GTCAGCTTCA GTCAGCTTCA 1000
 1001 TGATGGTGT ATCACCTACT ATCTAGACAC TGTGAAGGGC CGATTCACCA TTTCAGAGAA CAATGCCAAG ARACCCCTGT ACCTGCAAT GAGGAGCTTG 1100
 1101 AGCTCTGAGG ACACAGCTT GTTTTACTGT GCAGAGCACC GTCGGGGCTA CTTTCTATG GACTACTGGG GTCCCTCTGAGG TAGCCGGCTG 1200
 1201 GTAGGAATGG CCTCTCCAGG TCTTTTTTAAATCTTGTAA ATGGAGTTT CTGAACATTCG CAGACTTCA ATTATCTCCA CATTCTTGTAA AACACTGAAAT CTGTGTGTAT GTTGTGTGT 1300
 1301 AGAGAAATGG GGATTTAAC TCTCTTGGGA TCTOAGAGCC TTAGGACAG GCTAAACAA TCCATATGGT GGAGGGGGAG TTGGGGCTGT AGTTGGAGAT 1400
 1401 GAGTCCCTGG ATGATGGGT AGGGACTTGT GAGGCTCATTT TGAGGGAT GTCAGCTGAC AGCATTATA CAGTATCCGA TGCATAGGGAA CAAAGAGTGG 1500
 1501 TTTCAGTTT TAGTATAAA GCTTGTGGCA ATGTTCCACA CTAGTTCTG TGAAACCTCA TTGTGTGGAG GGAGGAGCTGT CTTAAATGT GAGGGGGTTT CTAGTGCCT 1600
 1601 AGTGGGGCAC TTTCCTTTCGA TTGTGGGGAA ATGTTCCACA CTAGTTCTG TCTGCAAAAGG TCCAGAGGG TCTGTGTGGAG CTCAGCTTTC AAAGGAACAC AGAAATGATGT 1700
 1701 AGAAGGGCAT CTAGCCTCGG TCTCAAAAGG GTAGTTGTGTG TTCTAAGTGG GTCTGTGTGG GTCTGTGTGG AATATGTT AAATCTGTGAG TTAAATGT GAGGGGGTTT TCAAGTACTC 1800
 1801 GTATGGAATA ATAGAAGATG TTGCTTTTAC TCTTAAGTGG GTCTGTGTGG GTCTGTGTGG TCTGTGTGG AGGTAACTTA AAGTTAAACG GAGGAATGGG AGTGTAGGCTC 2000
 1901 ATTTTTTAC ATGTCACAAA TTTCCTGTCAC TCAATTGAG GTCTGTGTGG GTCTGTGTGG TCTGTGTGG TCTGTGTGG AGTGGAGCTA AGTGGAGCTA AGTGGAGCTA 2100
 2001 TCTCTATACCC, TATTCAGAC TCTCTTAA CAPTAATAA TTAGTTAA AATATTTTA ATGATTTAA GCTATGTTGA GTCTGTGTGG AATATTTTA ATGATTTAA



LC_cG250 (HindIII-fragment in expression vector)

double strand sequencing

10.05.2001

4501 GAGATCTGGG TCTGACTGCA GTTACCGTGG TCTCTAGAC GTTAACTGG GAGATTCGGG GGGGATCAGG AAATGAGGA CTCAGGGATA GAAAGGTCT 4600
4601 GAAGTCAGT TCAGCTCCTA AAATGGATGT GGAGGCAAC TTGAGATA AACTGAATGA CCCAGGGAT GAAACAGTGC AGATCAAAA GGGCCTGGA 4700
4701 GCTCTGAGAA CAGAAGGAGA GTCATCGTG TTGAGTTCC ACAAACTGT TCTTGAGTT TGCATAATAAA GTGGGATAGC AGAGTTGAGT GAGCCATAGG 4800
4801 CTGAGTTCTC TCTTTGTCT CTAAGTTT TAGACTACA AAAATCGTA GTATGTCCTG AAATAATCT TAACCTGTT GAAAGTATGA CTGCTTGCCA 4900
4901 TGAGATACC ATGGCTTGTCT GAATATTCAG AAGAGGTGTG ACTCTTATC TAAATTGT CACAMMTGT CAAATGAGA GACTCTTAGA AAACGAGTCC 5000
5001 TTGACAGACA GCTCAGGGG TTCTTCCT TTGTCTCATT TCTACATGA AGTAATTG AAATGATCTT TTATTTATAA ATAGTAGAA TACAGTGGG 5100
5101 TTGAACTAT ATGGTTTAAT GGGCACGGTT TTGTAAGACA TTGGCCCTT TGTTTTCCTA GTTATTACTC GCTTGTAATT TTATATCGGC AGCAATGGAC 5200
5201 TGAAACGGTC CGCAACCTCT TCTTACAAC TGGGTGACCT CGGGGTGTG CCAGGCCATT GGGGTTAAC TTGCGCGCTAA GGGCCGTGTG AACCCCGAG 5300
5301 GTAGCATCCC TTGCTCCGG TGGACCACTT TTCTGAGGA CAGTGATAGG AACAGAGCCA CTAATCTGAA GAGAACAGAG ATGTGACAGA CTACACTAAT 5400
5401 GTAGAAGAA CAAGGAAAGG GTGACTTATT GAGATTCA GAAATAAT GCATTATTA TTATATTCCC TTATTTATTG GGAATTAGAA 5500
5501 AGGGCATAAA CTGCTTATC CAGTGTATA TTAAAAAGCTT TTGTTTTC AGTGCTA 5557

Figure 3B:

LC_cG250 (HindIII-fragment in expression vector) double strand sequencing 10.05.2001

O I P E
OCT 30 2003
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U.S.A.

1 AATTCCAAGC TTGTATCTT CAGATCCAGG AAAGCCACCA CCATAATCAA ACAGATACAT GCTGAAACCA ACTTCCTTC TTAATCTAAA TGCACAGCG 100
101 GCATCTGACA CTGGCTGCAT GAAGGTCTCA GGTCAATACT TCCACTACAC ACATGGAAAGC TGACACCATT GACGTCAATA TTGAGCTCTT TTGCCCATTT 200
201 CAGGGGAGA CTGGCTGGTT TGAGTGTTGC ACCAGACTTA ACACCAAGTC GACAAACTGCA TTGGAATCA TCTGTGACAA TCCACAAAAA CAACTTTGTG 300
301 TTACATGTG CTCTGACGAC ATTCAATACT TCATTCACT GTCAAAAGTC ATCATCTGG CTTCATTACT. GGCAGCATAC TTGATTGAG ACATCTGTT 400
401 ACAAAATGT GCATAGTG CCATGGTAA CCCTCTCTGG AGGAAACCA AGCCCCGTT CCAACTGAT TTCAAGCTT CTTGCAAGT ACCATCTGT ACCATAGCA 500
501 GCTAGGGGT TAACATGGC TCTGTTGCC TTACACTTGA CTGACACAAA AGGAAATAACA TTGGAAAGAG CTTTAGCCA CCTCAGATGC TCTCTTTAGAA 600
601 TGTCTGTGAG GTCCGGAACC TAGAAAGAG AGACTCTATTG TTATATTG TGTTAGAT GTCTCTTAGCA CTAAAGCCAC CATCTATGAT ACAGGAGTCA 700
701 AACTCTTCCT TAGTATAGCT GCTCATGCTT CTCCATGTGC CTACAGAAA CCTAGACATG GAAATTAATTTT ATTGCCAGCC CCTTACAAGG TCAACTTATC 800
801 CAAGGAACCTG GAATGGAGAC TCCITGAAAT GTTGGAAACA CTACAGGAC AGGGTCAAGA CTGGCTGGAC ACATGGAGAC ACTGAATCTT GAAGGACACT 900
901 TAGCTGTCTG TGTCTCATC ATGTCTACTG ACCTGAGGT GCACCAAGCT GCCTACTGAG GGAGGACTGT GGGGGTGTGC GCAGGAACCTG ACAATTCTCC 1000
1001 ACAATTCTCT TACTGCCCA CTCTAAACTC TTCTCTCTCTC CATCTCTCTC TTTCTTCCTCTT CTCCCCTCTT CACTACTTTT TCCCTTTCTT 1100
1101 CTTTCCACT TCCCTTCTC TTCTCTTCTT CTTCTCTT CTTCTCTT CTTCTCTT CTTCTCTT CTTCTCTT CTTCTCTT CTTCTCTT CTTCTCTT 1200
1201 TAAAATCTAT CTGTGATTT TCTTAAATT ATTAAATT GTTACACTC CATATTTAT TCAACCCCTC CATCTCTCTA CTGGCTPACA TACCATCTCT 1300
1301 CCTTCCACA CCCCTGTC CACATGGAT CTGCCACCTC CCATGCCACC TGACCTCTCA TCTCCCTAGG :GCATCTAGTC TCTTGGGCT TAGATGATC 1400
1401 ATTCCTGAGT GAACAGAT CCAACATCC TCTGCTTATAT GTGTGTTGGT GGCTCTATAG CAGCTGGGT ATGCTGGCT TCTTGGTGCCT CAGTGTGTTA 1500
1501 GAGGTCTGC GGGTCACT TAACTGAGAT TTGTGAGAT TTGTGGACCT CCTCAGGTC TTTCTGCTT TCCCTGATC AACAAACAGGG TTCAATGTTT CTGTCTATG 1600
1601 GTGGGTGCA AATATCTGCA TCTGACTCAG CTGGCTTATGG GGTCTTCTGG AGTGCAGTCAGT GGTCTTCTGG ACCCCCTCC 1700
1701 TAGTGTCAAG CGTGGGACT GCCCTTGAC CTGGATTCA TTTGGACCT GTGCTGGACT CTCCTCTCC CTCATTTAT GCCCTGTCCTT CCTGGTGGAA GTGGGGCTCTA 1800
1801 TTCTTTGAGA CAGGAACAA TATGGTCAG AGTGTGAGT GTGGAAATGGC GTGCTGGAC CTCGGCTCCC CTCCATCTGT ATCCCTGTAA 1900
1901 TAAGTCTCCA CTCCCTACTG TGGCCTTGTGAT CTCCTCTTG AGTCCCTGAG GTCTCTCACC TCCCAGGTT CTGGTGGTC CTCAGTCTT 2000
2001 CCTAACCTCCC CAGGTGGCT CTTGACAGAC TTCTGCTGGC CCCCTAGTGT TCCCTCCTT TCCCTCACCC AATATCTGAT TGGATGGAA GCCTGTCATG 2100



HC_cG250 (EcoRI-fragment in expression vector)

double strand sequencing

08.05.2001

2101 TCAGAACCG AACACCTGCA GCAGCTGGCA GGAAGCAGGT CATGTGGCAA GGCTATTGG GGAAGGGAAA ATAAAAACAC TAGTTAACT TGTAGGCTGG 2200
2201 GTTTGAGAA GTGGTTTGA AACACTCTGT CCAGCCCCAC CAAACCGAAA GTCCAGGCTG AGCAAAACAC CACCTGGTA ATTTCATTT CTTAATAAG 2300
2301 TTGAGGATTG AGCCGAAACT GGAGAGGCC TCTTTAACT TATTGAGTC AACCTTTAA TTTCAGCTGA AGTAGTTCA GTTCCCCAA ACTTAAGTT 2400
2401 ATCGACTCTT AAAATGTTT TAGAATTAT T 2431



LC_cG250 (HindIII-fragment in expression vector)

double strand sequencing 10.05.2001

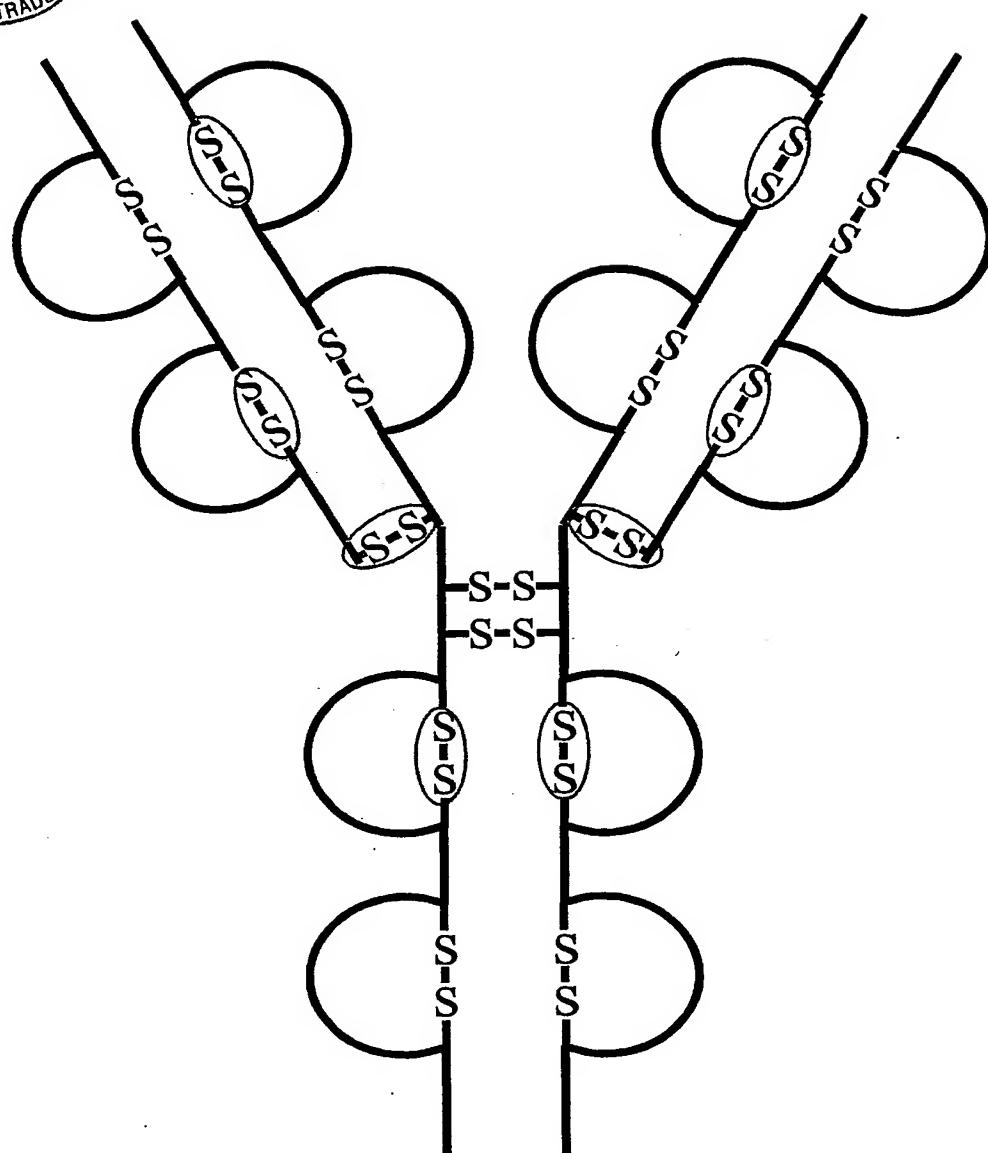


Figure 4

Schematic figure of an antibody. Disulfide bonds detected by mass spectrometry after enzymatic digest in WX-G250 are labeled by ovals.

Figure 5

Characterization of WX-G250 according to MALDI-PMF (Trypsin, LysC, AspN, GluC, and BrCN) in reflector and linear mod



Light Chain:

DIVMTQSQRF MSTTVGDRVS ITCKASQNVV SAWAWYQQKP
GQSPKLLIYS ASNRYTGVPD RFTGSGSGTD FTLTISNMQS
EDLADEFCQQ YSNYPWTFGG GTKLEIKRTV AAPSVFIFPP
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ
ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC

Heavy Chain:

DVKLVESGGG LVKLGGSLKL SCAASGFTFS NYMSWVRQT
PEKRLELVAA INSDGGITYY LDTVKGRFTI SRDNAKNTLY
LQMSSLKSED TALFYCARHR SGYFSMDYWG QGTSVTVSSA
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW
NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP
SVFLFPPKPK DTLMISRTP ETCVVVDVSH EDPEVKFNWY
VDGVEVHNAK TKPREEQYNSRVSVLTU LHQDWLN⁺GKE
YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSRDEL
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWO QGNVFSCSVM HEALHNHYTQ
KSLSLSPCR

Reflector mode

Additional information
from linear mode spectra

C Detected bridged cysteins (reflector mode)

(C) Detected bridged cysteins (linear mode)

C: Cysteine not determined as bridged cysteins

K: Heavy chain partially lacks C-terminal lysine



Confirmed glycosylation site



Confirmed deamidation sites



Light chain:

DIVMTQSQRF MSTTVGDRVS ITCKASQNVV SAVAWYQQKP GOSPKLLIYS
ASNRYTGVPD RFTGSGSGTD FTLTISNMQS EDLADFFCQQ YSNYPWTFGG
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLN^NFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC

Heavy chain:

DVKLVESGGG LVKLGGSLKL SCAASGFTFS NYYMSWVRQT PEKRLELVAA
INSDGGITYYY LDTVKGRFTI SRDNAKN^TLY LQMSSLKSED TALFYCARHR
SGYFSMDYWG QGT^SVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFP^AVLOQSSG LYSLSSVVTV PSSSLGTQTY
ICNVN^HKPSN TKVDKKVEPK SCDKTHTCPP CPAPELLGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHN^AK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSRDEL TKNQVSLTC VKGFYPSDIA VEWE^SNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSC SVM HEALHNHYTQ KSLSLSPGK

Figure 6: LC-MS and LC-MS/MS of tryptic digest of cG250